Phytic acid in wheat bran affects colon morphology, cell differentiation and apoptosis

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Wheat bran (WB) and its component phytic acid (PA) have both been shown to decrease early biomarkers of colon carcinogenesis, i.e. the PCNA labeling index of cell proliferation and certain aberrant crypt foci parameters. However, it is not known how WB and PA alter other biomarkers of colon cancer risk, such as rate of apoptosis and degree of differentiation, or how they affect colon morphology. Thus, the objectives of this study were to determine the effects of WB on these parameters, to see if PA contributes to these effects and whether there is a difference between endogenous and exogenously added PA. Five groups of azoxymethane-treated male Fischer 344 rats were fed a basal control diet (BD) or BD supplemented with either 25% wheat bran, 25% dephytinized wheat bran (DWB), 25% DWB plus 1.0% PA or 1.0% PA for 100 days. The WB, DWB and PA diets significantly increased the rate of apoptosis and cell differentiation in the whole crypt and the top 40% of the crypt. The WB, DWB and PA diets also significantly increased cell apoptosis in the bottom 60% of the crypt, while all the treatment groups significantly increased cell differentiation versus the BD group in the bottom 60% of the crypt. In addition, the WB, DWB and PA diets decreased the number of crypts per millimeter of colon, while the DWB and PA diets also decreased crypt height measured as number of cells. It is concluded that WB, partly due to its dietary fiber and endogenous PA, and exogenous PA when added to a low fiber diet can increase cell apoptosis and differentiation and favorably affect colon morphology.

Introduction

Numerous animal studies have consistently shown that wheat bran (WB) has a colon cancer protective effect at early (1) and late stages of tumorigenesis (2–7). This effect of WB has been attributed to both its high dietary fiber and phytic acid (myo-inositol 1,2,3,4,5,6-hexakis-dihydrogen phosphate; PA) content (1). Many of the proposed protective mechanisms of WB and PA action, such as decreased transit time (8), increased bulk (9) and fermentation (10), appear to be shared. In addition, it has previously been shown that WB and PA diets protect against early biomarkers of colon cancer by reducing the proliferating cell nuclear antigen (PCNA) labeling index (LI) of cell proliferation (1). However it is still unclear whether these diets also affect other important processes involved in maintenance of the colonic epithelium, i.e. cell differentiation and apoptosis, which need to be considered in the evaluation of colon cancer risk. The importance of these factors is highlighted by the recent suggestion that their measurement may have greater prognostic significance in the assessment of dietary effects on tumor incidence and colon cancer risk than measurements of cell proliferation alone (11).

In the colon carcinogenic process the interplay between cell proliferation, differentiation and apoptosis is very important (12). It is clearly conceivable that increased or uncontrolled proliferation, failure to properly differentiate and a delay in apoptosis may collectively lead to changes in colonic and crypt morphology and architecture, increased cell bulk and a heightened probability of ‘fixation’ of any mutations that may be present. The importance of apoptosis, as a means of eliminating damaged cells and in the maintenance of colon tissue homeostasis (the balance between cell proliferation and cell death and the maintenance of constant crypt length), is slowly coming to light. In fact, inhibition of apoptosis has been shown to play an important role in the genesis of colon adenomas and carcinomas (13) and has been suggested to be a risk factor for colon cancer (14). If colonic epithelial cells no longer respond to DNA damage by undergoing apoptosis, then mutations possibly leading to colon cancer may be acquired and fixed through further proliferation. Apoptotic death may be initiated, inhibited or modulated by the presence or absence of certain endogenous stimuli, such as growth factors, hormones and cytokines (12), or exogenous factors, such as drugs, ionizing radiation (15) and, most importantly, diet (16,17).

Similar to apoptosis, a delay in or failure of differentiation has been proposed to be important in assessing dietary colon cancer risk (18). Both the microfloral environment of the colon (19) and the diet (11,18), which can affect the flora, have been shown to modulate the degree of cell differentiation in the colon. In addition, feeding of some known differentiation-inducing agents has been shown to significantly decrease some aberrant crypt foci (ACF) (early biomarkers of colon cancer risk) parameters (20). The more strongly colon cells are stimulated to differentiate, the less likely they are to proliferate and, hypothetically, the higher their rate of apoptosis.

The intensity of Dolichos biflorus agglutinin (DBA) lectin staining and the ratio of sulphomucins (SUM) to sialomucins (SIM) are properties of glycoconjugate alterations in colon mucosal cells associated with their stage of maturation; they have been used as valid markers of the degree of colonic mucosal differentiation in a number of human (21) and animal (11,16,18) studies. Both DBA lectin staining intensity and an increased SUM:SIM ratio are indicative of increased cellular maturity, since decreased DBA lectin binding and SUM
production have been observed in abnormal, preneoplastic and neoplastic colon tissues as compared with normal mucosa (22–26). Thus, techniques assessing changes in types of mucin produced and their carbohydrate profiles can accurately display the state of differentiation in the colon.

Despite the vast amount of information on the protective effects of WB and PA against colon cancer, there is very little known about how these diets affect colonic crypt morphology, rate of apoptosis and degree of differentiation. Since changes in apoptosis along with a lack of or delay in differentiation are important processes in the promotion of colon carcinogenesis, our objectives were to determine whether these factors play a role in the previously observed early colon cancer biomarker inhibitory effects of WB (1), whether PA is the component of WB responsible for these effects and whether there is a difference between endogenous PA and pure exogenous PA added to the diet.

Materials and methods

Experimental design

Seventy-five male 40-day-old Fischer 344 rats (Charles River Inc., Montreal, Canada) were maintained in individual stainless steel cages at an ambient temperature of 22–24°C on a 12 h light/dark cycle. They were acclimatized for 2 weeks on the AIN-93G basal diet (BD) (27) and then injected i.p. with 15 mg/kg body wt of the colon carcinogen azoxymethane (Sigma Chemical Co., St Louis, MO). One week later they were randomized into five groups with 15 rats/group, such that the mean weight of each group was equal. They were fed ad libitum either the BD (BD group) or BD supplemented with either 25% WB (WG group), dephytinized WB (DWB group), 25% DWB plus 1.0% PA (DWBPA group) or 1.0% PA (PA group). All diet ingredients were ordered from Dyets Inc., except for WB (King Milling Co., MI). The 25% WB level was chosen so as to provide a 1.0% level of PA in the diet. All the diets were based on the AIN-93G diet (27) containing 39.75% corn starch, 20.00% casein, 13.20% dextrinized corn starch, 10.00% sucrose, 7.00% soybean oil, 5.00% dietary fiber, 3.50% mineral mix, 1.00% vitamin mix, 0.30% l-cystine, 0.25% choline bitartrate and 0.0014% t-butyldihydroquinone. The WB-containing diets were corrected for the protein, fat, fiber and moisture content contributed by the added WB. The WB was dephytinized according to the method of Morris and Ellis (28), i.e. by activating the bran’s natural phytases by incubating a 15% dispersion in deionized water at 37°C for 18 h in a pasteurization vat followed by freeze drying. The PA content was determined to be 4.10% for the dephytinized WB and 0.12% for the DWB by the Association of Official and Analytical Chemists method (29) and 4.42 and <0.2% by HPLC (30). The HPLC analysis also showed that the levels of low molecular weight PA substances in the WB and DWB were negligible. The level of added PA in the DWBPA diet was adjusted for the residual PA in the dephytinized wheat bran such that the overall concentration of PA in the diet was 1.0%.

The food cups were supplied with fresh food diet every 2 days. Fresh diet was prepared biweekly and stored at –20°C. In use diets were refrigerated at 4°C. After 100 days treatment the rats were killed by CO2 gassing. The distal colon sections were wrapped in ‘swiss roll’ style, embedded in paraffin blocks and sliced at 5 µm thickness with two sections from each rat on each slide. Adjacent slides from 8–10 animals/group, chosen at random, were used for the measurement of rate of apoptosis, degree of cell differentiation and colon morphometry. All slides were coded and the observer was blind as to the dietary groups to which the animals belonged. For each analysis a minimum of 24 crypts/colon were counted. All counting was performed at 400× magnification. Only whole longitudinally sectioned crypts that showed the entire column length from the lumen down to the muscularis mucosa were counted. Incomplete crypts or those with more than two missing cells were not counted.

Measurement of apoptosis

The rate of apoptosis was measured via the terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL) technique using the in situ cell death detection kit from Roche Molecular Biochemicals (mannheim, Germany), which labels DNA strand breaks produced during apoptosis. Briefly, the tissue sections were deparaffinized, rehydrated and washed with phosphate-buffered saline (PBS). The slides were then microwaved for 5 min on low power in citric acid buffer, allowed to cool, washed twice in PBS, treated with proteinase K (20 µg/ml in 10 mM Tris-HCl) for 15 min at room temperature, rinsed twice with PBS, incubated with 3% H2O2 in methanol for 10 min at room temperature and again rinsed twice with PBS. They were then incubated for 60 min at 37°C in a humid chamber with 50 µl of TUNEL reaction mixture consisting of terminal deoxynucleotidyl transferase and the nucleotide, rinsed three times with PBS, incubated for 30 min at 37°C in a humid chamber with converter-POD peroxidase-conjugated antibody and rinsed three times with PBS. Slides were incubated for 10 min at room temperature with 100 µl of diaminobenzidine tetrahydrochloride (DAB) (Vector Laboratories, Burlingame, CA) substrate solution, counterstained with hematoxylin for 20 s, rinsed in distilled water and then tap water and mounted with Permount. Only the apoptotic morphology (condensed chromatin, fragmented nuclei and/or apoptotic bodies) of TUNEL positive cells was scored. The apoptotic index (AI) was calculated as the percentage of scored cells for the whole colon and top 40% and bottom 60% of each crypt.

Measurement of cell differentiation: lectin histochemistry

The method was modified from that of Boland (23). The tissue sections were deparaffinized and rehydrated. Endogenous peroxidase activity was blocked by immersing the slides in 0.3% H2O2 in methanol for 10 min with a subsequent double wash in distilled water. The slides were then treated with a solution of 0.025% Triton in PBS. Biotinylated DBA (10 µg/ml) was placed on the tissue sections and incubated in a humid chamber for 30 min. The slides were washed with PBS, then treated with streptavidin complex (Dako, Carpenteria, CA) for 30 min and washed three times with distilled water. The color was developed by adding 100 µl of 0.5 mg DAB/ml PBS with the addition of 0.015% H2O2 immediately prior to staining for 7 min. The slides were washed three times with distilled water, counterstained with hematoxylin for 20 s, rinsed in distilled water and then tap water and mounted with Permount. To test staining specificity, 0.2 M N-acetyl-d-galactosamine (Sigma Chemical Co.), which inhibits lectin binding, was added to a control slide for each run.

The lectin scoring index was calculated according to the method developed by Chang et al. (11). This method determines a score that ranges on a scale from 0 to 4 based on the percentage of cells stained and also the intensity of staining. For example, crypts with a similar percentage of positively stained cells but a different intensity of stain color would receive different scores, with a higher score indicating a greater number of stained cells and a greater intensity and, hence, a higher degree of differentiation.

Measurement of cell differentiation: determination of sulpho- and sidomucins

The high iron diamine Alcian blue method (31) was used. Briefly, tissue sections were deparaffinized and rehydrated. Endogenous peroxidase activity was blocked by immersing the slides in 0.3% H2O2 in methanol for 10 min with a subsequent double wash in distilled water. The slides were then immersed in high iron diamine solution (20 mg N,N’-dimethyl-p-phenylenediamine and 120 mg N,N’-dimethyl-m-phenylenediamine in 50 ml of distilled water plus 1.4 ml of 20% ferric chloride; all from Sigma Chemical Co.) in a covered slide pack protected against light for 18 h at room temperature. The slides were then rinsed three times in distilled water, stained with 1% Alcan blue (Sigma Chemical Co.) in 3% acetic acid for 30 min and rinsed three times in 80% ethanol and once in distilled water. Dark brown staining of the crypt goblet cells implied SUM production, bright or dark blue staining indicated predominantly SIM production, while a mixture of brown and blue staining indicated the production of both SUM and SIM. Since these were distal colon sections, it was anticipated that SUM production would be the predominant type of staining observed. The number of cells displaying each type of mucin were counted in each crypt and the ratio of the number of SIM- to SUM-producing cells calculated.

Colonic mucosal morphometry

The tissue sections were deparaffinized, rehydrated and stained with hematoxylin and eosin. Crypt cell height, defined as the full height of the crypt in number of cells, and crypt density, defined as the number of crypts per mm2 of colon determined with a micrometer, were measured. At least 18 measurements of crypt density were taken at various parts of the distal colon. Colon length was determined at the time of death and the data used to calculate ratios of colon length to crypt height and to crypt density.

Statistical analyses

All data were analyzed by one- or two-way analysis of variance followed by either the Tukey’s pairwise or Dunn’s pairwise non-parametric multiple comparisons tests using the SigmaStat statistical software package (Jandel Scientific, San Rafael, CA).

Results

Apoptosis

Compared with BD, a significant increase in AI was seen in the WB, DWB and PA groups in the whole crypt and the

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than any of the treatment groups (Table III). The ratio of SIM scores in the whole, top 40% and bottom 60% of the crypt were significantly different, *P* < 0.05 by one-way ANOVA followed by either Tukey’s pairwise parametric or Dunn’s pairwise non-parametric multiple groups comparisons tests; *P* values in the table indicate results for a two-way ANOVA on the effects of fiber, PA or their interaction using the BD, WB, DWB and PA groups. NS, not significantly different. *n* = 10 rats per group.

| Table I. Apoptotic indices in the distal colon of the various dietary groups |
|-------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Diet group                   | BD              | WB              | DWB             | DWBPA           | PA              |
| Whole crypt                  | 1.84 ± 0.24<sup>a</sup> | 5.84 ± 0.54<sup>a</sup> | 5.44 ± 0.69<sup>a</sup> | 4.34 ± 0.46<sup>b</sup> | 6.59 ± 0.54<sup>a</sup> |
| Top 40%                      | 3.29 ± 0.51<sup>b</sup> | 8.71 ± 0.66<sup>a</sup> | 8.92 ± 1.37<sup>b</sup> | 7.76 ± 0.87<sup>a</sup> | 7.86 ± 0.65<sup>a</sup> |
| Bottom 60%                   | 0.80 ± 0.24<sup>a</sup> | 2.94 ± 0.64<sup>b</sup> | 2.57 ± 0.39<sup>b</sup> | 1.61 ± 0.15<sup>c</sup> | 5.28 ± 0.51<sup>a</sup> |

Values are means ± SEM. Apoptotic index = (no. of apoptotic cells/total no. of cells)×100. Values with different superscripts within a row are significantly different, *P* < 0.05 by one-way ANOVA followed by either Tukey’s pairwise parametric or Dunn’s pairwise non-parametric multiple groups comparisons tests; *P* values in the table indicate results for a two-way ANOVA on the effects of fiber, PA or their interaction using the BD, WB, DWB and PA groups. NS, not significantly different. *n* = 10 rats per group.

| Table II. Lectin scores in the distal colon of the various dietary groups |
|-------------------------------|-----------------|-----------------|-----------------|-----------------|
| Diet group                   | BD              | WB              | DWB             | DWBPA           |
| Whole crypt                  | 0.94 ± 0.11<sup>a</sup> | 2.52 ± 0.20<sup>a</sup> | 2.30 ± 0.21<sup>a</sup> | 2.59 ± 0.15<sup>a</sup> |
| Top 40%                      | 1.03 ± 0.12<sup>b</sup> | 2.65 ± 0.19<sup>a</sup> | 2.15 ± 0.15<sup>b</sup> | 2.61 ± 0.20<sup>a</sup> |
| Bottom 60%                   | 0.97 ± 0.14<sup>a</sup> | 2.37 ± 0.23<sup>a</sup> | 2.39 ± 0.25<sup>a</sup> | 2.63 ± 0.16<sup>a</sup> |

Lectin scores range from 0 to 4 and are calculated depending on percentage of cells stained and staining intensity (11). A higher score indicates a greater degree of differentiation. Values are means ± SEM. Values with different superscripts within a row are significantly different, *P* < 0.05 by one-way ANOVA followed by either Tukey’s pairwise parametric or Dunn’s pairwise non-parametric multiple groups comparisons tests; *P* values in the table indicate results for a two-way ANOVA on the effects of fiber, PA or their interaction using the BD, WB, DWB and PA groups. NS, not significantly different. *n* = 8 rats per group.

| Table III. Type of mucin production in the distal colon of the various dietary groups |
|-------------------------------|-----------------|-----------------|-----------------|-----------------|
| Diet group                   | BD              | WB              | DWB             | DWBPA           |
| Percent SIM cells             | 26.22 ± 2.23<sup>a</sup> | 6.20 ± 1.00<sup>b</sup> | 8.36 ± 1.84<sup>b</sup> | 5.90 ± 1.01<sup>b</sup> |
| Percent SUM cells             | 38.44 ± 5.0<sup>a</sup> | 60.38 ± 2.67<sup>a</sup> | 57.04 ± 3.16<sup>a</sup> | 62.88 ± 3.55<sup>a</sup> |
| Percent SIM + SUM cells       | 35.34 ± 3.90<sup>a</sup> | 33.42 ± 2.11<sup>a</sup> | 34.60 ± 1.93<sup>a</sup> | 31.23 ± 2.66<sup>a</sup> |
| SIM-SUM                      | 0.90 ± 0.22<sup>a</sup> | 0.11 ± 0.02<sup>a</sup> | 0.17 ± 0.05<sup>a</sup> | 0.10 ± 0.02<sup>a</sup> |

Values are means ± SEM. Values with different superscripts within a row are significantly different, *P* < 0.05 by one-way ANOVA followed by either Tukey’s pairwise parametric or Dunn’s pairwise non-parametric multiple groups comparisons tests; *P* values in the table indicate results for a two-way ANOVA on the effects of fiber, PA or their interaction using the BD, WB, DWB and PA groups. NS, not significantly different. *n* = 8 rats per group.

bottom 60% of the crypt (Table I). AI was also increased by DWBPA in the whole crypt and bottom 60% of crypt, but it did not differ significantly from any of the groups except the PA group in the bottom 60% of crypt. All the treatment groups had a significantly higher AI than the BD group in the top 40% of the crypt. Analysis via two-way ANOVA showed a significant effect of dietary fiber, PA and their interaction in the whole crypt and the top 40% of the crypt, but not in the bottom 60% of the crypt.

**Colon cell differentiation**

All the dietary treatment groups had significantly higher lectin scores in the whole, top 40% and bottom 60% of the crypt (Table II). In addition, two-way ANOVA showed a significant effect of dietary fiber, PA and their interaction in the whole crypt, the top 40% and the bottom 60% of the crypt.

The BD group showed a significantly much higher production of SIM and lower production of SUM in the distal colon than any of the treatment groups (Table III). The ratio of SIM to SUM showed a similar pattern, being significantly higher than the treatment diet groups (Table III). There were no differences in the percentage of cells producing a mixture of SIM and SUM amongst the various dietary groups. An effect of fiber, PA and their interaction was observed for the percentage of cells producing SIM and for the overall ratio of SIM to SUM. An effect of fiber and PA, but not their interaction, was observed for the percentage of cells producing SUM. No significant effects of fiber and PA were observed for cells producing a mixture of the types of mucins.

**Mucosal morphometry**

Although all the treatment groups had reduced crypt height, measured in number of cells, only the DWB and PA groups were significantly lower (Table IV). Two way ANOVA did not show an individual effect of dietary fiber or PA but did show a significant effect of their interaction on reduction of crypt cell height. The WB, DWB and PA groups all had significantly lower crypt density, defined as the number of crypts per millimeter of colon. A significant effect of both fiber and PA was observed for crypt density. There were no significant differences observed for colon length, but there was a significant effect of fiber affecting colon length. There
were no significant differences amongst the various diet groups in the ratio of crypt density to height. The ratio of colon length to crypt cell height was significantly higher in the DWB and DWBPA groups than the BD group, with a significant effect of fiber and interaction of fiber and PA, but not PA alone, being observed. The ratio of colon length to crypt density was significantly higher in the WB and DWB groups than the BD group with only a significant effect of fiber being observed using the two-way ANOVA.

Discussion

This study has shown that WB, DWB, DWBPA and PA diets can increase cell apoptosis and differentiation and affect colon morphometry. These observations are particularly important since, coupled with the previously reported concomitant reduction in rate of cell proliferation caused by these diets (1), they provide a mechanism whereby WB and PA may exert their protective effects on early biomarkers of colon carcinogenesis (1).

It has previously been shown that the rate of apoptosis and degree of differentiation are important prognostic indicators of colon tumor development and colon cancer risk and may be even more predictive than measurements of the rate of cell proliferation (11). It is probable that all these three mechanisms function together to limit the growth and abnormal expansion of cells in the colon. Apoptosis is key for the timely death of terminally differentiated cells or cells with damaged DNA. Suppression of apoptosis may lead to an increased cell lifespan or to an accumulation of genetic damage (32,33), while the converse may be true for promotion of apoptosis. Since it has recently been suggested that disruption of apoptosis may be an early event in carcinogenesis (34), the observation in this study that WB, DWB, DWBPA and PA can all significantly increase the rate of apoptosis versus the control group at 100 days suggests that this is an important mechanism in their protective effects against colon cancer. Compher et al. (35) have recently shown an increase in apoptosis at the initiation stages of colon carcinogenesis but not at 56 days post-initiation with 20% WB feeding. However, their results were limited by the small size of the colon section studied and the small number of crypts counted (10, versus 24 in this study).

In this study the WB diet was used to show the effects of both WB fiber and endogenous PA, while the DWB diet was designed to show the effect of WB fiber without endogenous PA and the PA diet was designed to show the effect of exogenous, pure PA without interference by WB fiber. Thus, it is of particular interest that there was no significant difference in rate of apoptosis or degree of differentiation between the WB, DWB and PA groups. This suggests that both WB fiber and PA, whether added exogenously or present endogenously (within the matrix of WB fiber), can affect apoptosis. In fact, our data show a significant effect of WB fiber, PA and their interaction on rate of apoptosis in the whole crypt and top 40% of the crypt.

It is unclear whether the mechanisms of this modulation are the same for WB and PA. Although it has been suggested that WB and PA share a number of the same proposed colon cancer protective mechanisms of action (e.g. physical dilution of gut contents, shortened transit times, alterations in mutagenicity of the intestinal contents and production of butyrate), PA from the diet may also participate in cellular inositol phosphate pools in colon cells and affect secondary messenger pathways (35,36), thus altering cell growth and gene expression. In fact, pure PA has been shown in vitro to inhibit growth and promote differentiation in a number of cancer cell lines (38–41). It may accomplish this by up-regulating expression of the p53 gene (42), which has been shown to be intimately involved in the type of apoptosis that occurs in times of cellular stress or genetic insult (43).

Colonic butyrate levels may be increased by both WB (44,45) and PA, by causing starch malabsorption leading to increased colonic fermentation and production of butyrate and other short chain fatty acids (46). Butyrate has been shown to induce differentiation (47,48) and also to promote apoptosis via a p53-independent pathway (49). The mechanism of butyrate-induced apoptosis in the colon may be related to expression of the bcl-2 gene, which blocks apoptotic cell death (12), or the bak gene, which is an apoptosis promoter (50). Hague et al. (51) suggested that butyrate may be able to induce apoptosis in the colon by either decreasing the levels of bcl-2 protein or by inducing expression of bak. Thus, increased production of butyrate due to WB and PA feeding, and its subsequent modulation of the bcl-2 or bak gene, may be responsible for the increased levels of apoptosis that we have observed in this study with WB and PA feeding. This may be particularly true for the PA diet, since it had the highest rate of apoptosis in the bottom 60% of the crypt, where bcl-2 is most highly expressed (12).

WB may also increase the rate of apoptosis via a more simple mechanism. It is known that detachment of cells from the top of crypts may induce apoptosis, since contact between colonocytes and the extracellular matrix is essential for their
survival (12). WB fiber has a significant sloughing ability and thus could induce more apoptosis by causing more cells to detach from or become less firmly attached to the tops of the crypts.

This study also showed that all the treatment diets could induce a greater state of differentiation in the colon. SUM are the predominant acid mucins in the normal distal colon of humans and rats (18,22). The ratio of SUM to SIM decreases in colon tumors, which have a lesser degree of cellular differentiation as compared with normal colonic mucosa (22), suggesting that this ratio is indicative of the state of maturation of colonic mucosa. Changes in types or ratios of mucins produced also affect lectin binding patterns. In fact, glycoconjugate modifications are early events in colorectal carcinogenesis (26), with a decrease in DBA lectin binding in less differentiated and more abnormal colonic tissues such as polyps and tumors and increased binding in normal colonic mucosa (23–26). Binding of DBA lectin to colonic glycoconjugates changes with their degree of maturation and differentiation, with lesser intensity of staining in the bottom sections of the crypt and increased intensity at the top of the crypt (52). It has been suggested that immature sugar side chains are produced in the lower regions of the crypt while cells that have moved up the crypt are more mature and have α-N-acetylgalactosamine residues, which react with DBA lectin, added to the end of the chain (53,54).

Our results indicate a shift towards an abnormal mucin profile or a less differentiated state for the control group (BD diet), while the WB- and PA-containing diets remained essentially normal, i.e. had a much higher level of SUM production. In general, it is thought that cells that are more differentiated will proliferate less (55). The fact that the WB and PA diets can maintain a higher degree of colon cell differentiation than the BD group may play a role in their observed colon cancer protective effects. We have previously reported (1) a concomitant decrease in the rate of cell proliferation along with decreases in ACF parameters in these same rats. Thus, the ability of these diets to reduce cell proliferation may be linked to their ability to induce more differentiation.

It is unclear how the treatment diets may be affecting differentiation, but pure PA in vitro has been shown to increase the differentiation of colon cancer cell lines (39–41). Likewise, butyrate, a product of WB fermentation, has also been shown to induce differentiation and reduce growth in several colon cancer cell lines (48,56). Increased production of butyrate may inhibit the activity of histone deacetylases (57), leading to histone hyperacetylation which may result in cell cycle arrest and more differentiation. Both PA, through the inositol phosphate pool and as a secondary messenger, and butyrate may directly participate in modulation of the expression of genes involved in cellular differentiation. The exact mechanisms of WB and PA action on induction of colon cellular differentiation require much further study.

All the treatment diets reduced the crypt height measured in number of cells, but only the DWB and PA groups were significantly lower. The BD group had significantly more crypts packed into 1 mm (crypt density) than the WB, DWB and PA groups. There was also a significant effect of WB fiber and PA on crypt density. Since these diets also decreased the rate of cell proliferation (1) and rate of apoptosis in this experiment, it is possible that the lower number of cells and crypts per millimeter are due to greater cell loss and less rapid replenishment of new cells. This is entirely possible, since Hong et al. (16) noticed a similar event: an increase in crypt cell height and number of crypts per millimeter of colon with a decrease in apoptosis and increase in cell proliferation. The ratio of crypt density to crypt height and the other ratios were calculated since they may give an indication of the 3-dimensional state of growth of the colon, e.g., how the diets were affecting crypt height in relation to colon length or number of crypts per millimeter. Presumably, similar to the principle behind the rate of cell proliferation being a colon cancer risk factor, the more growth there is within the colon the greater the cell bulk, the more probability there is that a mutation will occur and carry on to a tumor.

In conclusion, our results have shown that WB, due to its dietary fiber and endogenous PA component, and pure PA added to a low fiber diet can significantly increase the rate of apoptosis and degree of differentiation in the distal colon. They can also have favorable effects on colon morphology, reducing crypt cell height and crypt density. These results, coupled with the previously observed reductions in ACF and cell proliferation (1), show that WB, its fiber and PA can affect early events in colon carcinogenesis. However, the exact mechanisms of that modulation need further elaboration.

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